Contemporary genetic testing in inherited cardiac disease: Tools, ethical issues, and clinical applications

Clinical guidelines and position article

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Contemporary genetic testing in inherited cardiac disease: tools, ethical issues, and clinical applications

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Inherited cardiac diseases comprise a wide and heterogeneous spectrum of diseases of the heart, including the cardiomyopathies and the arrhythmic diseases in structurally normal hearts, that is, channelopathies. With a combined estimated prevalence of 3% in the general population, these conditions represent a relevant epidemiological entity worldwide, and are a major cause of cardiac morbidity and mortality in the young. The extraordinary progress achieved in molecular genetics over the last three decades has unveiled the complex molecular basis of many familial cardiac conditions, paving the way for routine use of gene testing in clinical practice. In current practice, genetic testing can be used in a clinically affected patient to confirm diagnosis, or to formulate a differential diagnosis among overlapping phenotypes or between hereditary and acquired (nongenetic) forms of disease. Although genotype–phenotype correlations are generally unpredictable, a precise molecular diagnosis can help predict prognosis in specific patient subsets and may guide management. In clinically unaffected relatives, genetic cascade testing is recommended, after the initial identification of a pathogenic variation, with the aim of identifying asymptomatic relatives who might be at risk of disease-related complications, including unexpected sudden cardiac death. Future implications include the identification of novel therapeutic targets and development of tailored treatments including gene therapy. This document reflects the multidisciplinary, 'real-world' experience required when implementing genetic testing in cardiomyopathies and arrhythmic syndromes, along the recommendations of various guidelines.

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Introduction

Inherited cardiac diseases comprise a wide and heterogeneous spectrum of diseases of the heart, including the cardiomyopathies and the arrhythmic diseases in structurally normal hearts, that is, channelopathies.[1] With a combined estimated prevalence of 3% in the general population,[1] these conditions represent a relevant epidemiological entity worldwide, and are a major cause of cardiac morbidity and mortality in the young. The extraordinary progress achieved in molecular genetics over the last three decades has unveiled the complex molecular basis of many familial cardiac conditions, paving the way for routine use of gene testing in clinical practice. According to the European Society of Cardiology classification, cardiomyopathies are divided into dilated (DCM), hypertrophic (HCM), arrhythmogenic right ventricular (ARVC), restrictive and unclassified, although in practice there may be extensive overlap between these phenotypes.[2] The hypokinetic non-dilated cardiomyopathy has been recently added to the prior major phenotypes.[3] The American Heart Association advanced a gene-based classification[4] so that HCM was viewed as a disease of the sarcomere[5] and ARVC as a disease of intercellular junctions, caused by mutations in genes encoding desmosomal proteins.[6,7] The genetics of familial DCM is far more heterogeneous: currently, DCM mutations have been described in genes encoding cytoskeletal, sarcomeric, desmosomal, nucleoskeletal, mitochondrial, and calcium handling proteins.[8]

Additionally, sarcomere mutations have been identified in association with more complex disorders of cardiac structure and function, including restrictive physiology and left ventricular noncompaction.[4] In the same manner, AQ4

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ARVC e DCM phenotypes can be a different expression of variants in the same genes (*LMNA*, *FLNC*, and desmosomal genes).[7] Channelopathies, generally caused by mutations in proteins constituting or regulating cardiac ion channels, include the long-QT syndrome (LQTS),the short-QT syndrome (SQTS), Brugada syndrome (BrS), and catecholaminergic polymorphic ventricular tachycardia (CPVT).[9] In this scenario, molecular cardiology has become an important tool to investigate the aetiology, pathogenesis, and development of inherited cardiac disease, and is beginning to change clinical practice. In current practice, genetic testing can be used in a clinically affected patient to confirm diagnosis, or to formulate a differential diagnosis among overlapping phenotypes or between hereditary and acquired (nongenetic) forms of disease.[10–15] Although genotype–phenotype correlations are generally unpredictable, a precise molecular diagnosis can help predict prognosis in specific patient subsets and may guide management.[16–18] In clinically unaffected relatives genetic cascade testing is recommended, after the initial identification of a pathogenic variation, with the aim of identifying asymptomatic relatives who might be at risk of disease-related complications, including unexpected sudden cardiac death.[19–21] Future implications include the identification of novel therapeutic targets and development of tailored treatments including gene therapy.

The document reflects the multidisciplinary, 'real-world' experience required when implementing genetic testing in cardiomyopathies and arrhythmic syndromes, along the recommendations of various guidelines.[15,22,23] We here address the analytical aspects of genetic testing, the complex field of attribution of pathogenicity for each mutation, the main aspects of genetic counselling, and various related ethical issues.

Genetic testing

Traditional methods

Since the introduction of genetic testing in the clinical practice of inherited cardiovascular diseases, the number and size of genes investigated have increased dramatically. Until 10 years ago, parallel mutation detection was largely performed by PCR-based techniques such as denaturing HPLC and high-resolution melting, both in the research setting and in clinical practice.[24,25] However, their sensitivity for variant detection range from 95% to as low as 80% and is highly dependent on adequate optimization of the technique.[26] Thus, although denaturing HPLC and high-resolution melting techniques have been widely used over the past years to perform genetic test in the setting of inherited cardiomyopathies,[27–29] they are now largely superseded.

Direct DNA sequencing, that is the process of determining the precise order of nucleotides within nucleic acid fragment, is the gold standard method for the detection of gene mutations. For the past 30 years the Sanger DNA sequencing technology, based on chain termination method, has been the dominant approach, and remains the test of choice in Mendelian diseases without genetic heterogeneity.[30] Sanger DNA sequencing, else known as 'first generation' sequencing, has enabled the identification of genes currently known to cause inherited cardiovascular diseases, demonstrating high accuracy and reproducibility. In the Heart Rhythm Society /European Heart Rhythm Association expert consensus statement on the state of genetic testing, Sanger sequencing was referred as a high-sensitivity method for the identification of mutations associated with cardiomyopathies and channelopathies.[15] However, neither scanning techniques nor sequencing are reliable in identifying medium/large insertions or deletions. Therefore, an additional technique, such as quantitative PCR or multiplex ligation-dependent probe amplification, is needed to assess gene dosage.[31–35]

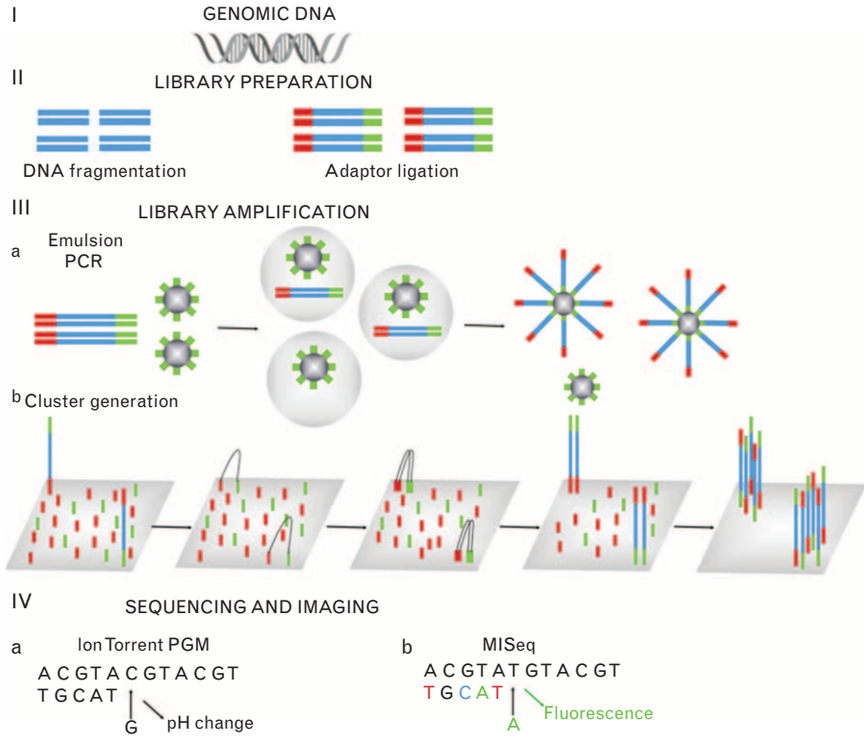
Massive parallel sequencing: the new standard

Despite its monumental accomplishments, Sanger sequencing can only analyse one DNA segment at a time and is thus laborious and time consuming, especially for genetically heterogeneous diseases such as cardiomyopathies/channelopathies. Time and cost limitations have therefore precluded its use for large-scale genome sequencing, stimulating the advance of powerful new technologies capable of delivering fast, less expensive, and accurate genome information.[36,37]

The commercial launch of the first massively parallel pyrosequencing platform in 2005 rapidly projected the field in a new era of high-throughput genomic analysis, currently referred to as next-generation sequencing (NGS). Although NGS platforms differ in their hardware configuration and sequencing chemistry, they share a common technical paradigm: massively parallel sequencing of clonally amplified or single DNA molecules that are spatially separated.[38–41] Through iterative cycles of polymerase-mediated nucleotide extensions (MiSeq, Ion Personal Genome Machine) or, in one approach, through successive oligonucleotides ligations (SOLiD), it is now possible to obtain sequence outputs in the range of hundreds of megabases to gigabases[38,39,42] (Fig. 1). In the past decade several NGS platforms have been developed and today the Life Technologies (Carlsbad, California, USA) Ion Torrent Personal Genome Machine and the Illumina (San Diego, California, USA) MiSeq and NextSeq are the most commonly used platforms.[43] Both allow for simultaneous interrogation of multiple genes in multiple samples by a single reaction. These strategies have proven accurate and effective in detecting mutations associated with Mendelian disease, both in the research and clinical settings.[41,44]

The advent of high-throughput sequencing has produced a number of commercially available sequencing options for genetic testing of cardiomyopathies, which

Fig. 1



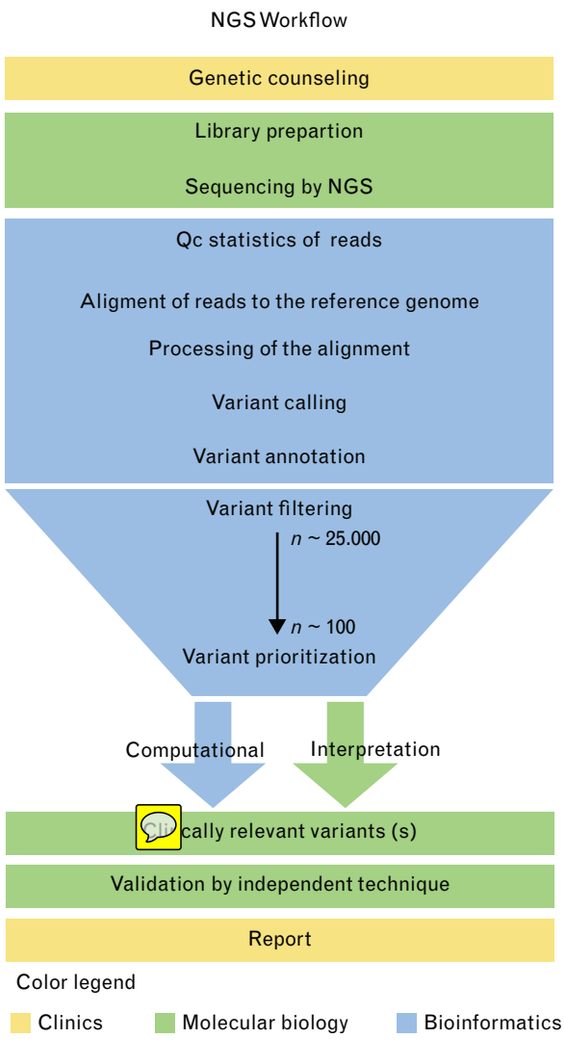
Workflow of next-generation DNA sequencing process. NGS, next-generation sequencing; PGM, personal genome machine. Reproduced from [41]. (I) Extraction of genomic DNA. (II) Ligation of specific adaptor (oligonucleotides) to fragments of the DNA to be sequenced. (III) Fragments amplification. (IIIa) The PGM system utilizes emulsion PCR to amplify single fragments onto microbeads, that will be loaded in the sequencing chip. (IIIb) The MiSeq system utilizes bridge amplification to form template clusters on a flow cell. (IV) Sequencing. (IVa) The PGM gets sequence information by detecting pH variations induced by the release of a hydrogen ion once a nucleotide is incorporated into a growing strand of DNA. (IVb) MiSeq is based on the detection of fluorescence generated by the incorporation of fluorescently labelled nucleotides into the growing strand. Once sequencing is complete, specific software process the raw data, to convert pH or fluorescence signals into actual sequence data. AQ8 AQ9 AQ10.

include: targeted panels that range from 5 to 20 genes, broad panels containing dozens of genes for a class of traits such as pan-cardiomyopathy or pan-arrhythmia (30–174 genes), whole exome sequencing (WES: about 20 000 genes), and whole genomes.[45] The advantage of more comprehensive tests is the potential for increased sensitivity of variant detection. The major drawbacks include costs, but mostly a huge increase in detection of variants of unknown clinical significance, enhancing the complexity of interpretation. Thus, a focused, targeted approach remains the cornerstone of testing in the clinical setting.[46]

Distinguishing pathogenic variants from background noise

NGS platforms generate millions of short (50–250 bp) sequence reads per run that must be processed by tailored bioinformatics pipelines. Figure 2 show the workflow used to sequencing and filtering the variants detected by NGS strategy. After library preparation and sequencing, bioinformatics analysis is performed. Bioinformatics analysis of data includes mapping of short sequencing reads to a reference genome by short read aligners,[47,48] processing of alignment through duplicated sequences removal, base quality recalibration and alignment correction,[49,50] variant calling,[49–51] and genomic and functional annotation of the variants.[52–56] Annotation is the collection of all available information to distinguish clinically relevant from common or private variants. Typically, tens of thousands genomic variants are identified by WES in a single patient. Annotation is the process that helps genomic analysts and clinicians to distil these huge amounts of data by using genomic and functional information collected in biological and/or clinical databases. The American College of Medical Genetics and Genomics has emphasised the most important criteria to establish causality of putative disease-causing mutations are minor allele frequency with a credible cut off of 0.01%, cooccurrence with disease, in-silico pathogenicity scores, and when possible familial cosegregation and functional assays.[57] Therefore, annotation tools identify which variants are reported in allele frequency databases, such as dbSNPs (https://www.ncbi.nlm.nih.gov), 1000 Genome Project (http://www.internationalgenome.org/), Exome Sequencing Project (http://evs.gs.washington.edu/EVS/),[58–60] or

Fig. 2



Schematic workflow of a standard genetic analysis by next-generation sequencing. NGS, next-generation sequencing; PGM, personal genome machine. After genetic counselling, patient's tissue sample is collected. Depending on the most appropriate protocol (targeted gene sequencing or whole exome sequencing), NGS libraries are prepared and sequenced through a next generation sequencers. Sequencing reads are evaluated through different quality measures. Reads are aligned to the human reference genome by a short read aligner. Resulting alignment is then processed for sorting, indel realignment, and base quality recalibration and eventually duplicates removal. Appropriate softwares can be used to identify single nucleotide variants and small insertion/deletions. Annotation of detected variants is performed by programs that compute amino acid coding changes and annotate variants to allele frequency, clinical, and functional prediction databases. To identify a small set of rare, possibly pathogenic, variants, filtering of annotated variants based on functional and allele frequency criteria is performed. Next, prioritization strategies are aimed at identifying clinically relevant variant(s). These can involve tailored interpretation of variants or the use of appropriate computational methods. Identified clinically relevant variants can be validated through independent molecular techniques, such as Sanger sequencing. Finally, a clinical report is prepared.

Exome Aggregation Consortium[61] (http://exac.broadinstitute.org/),Genome Aggregation Database,[62] (http://gnomad.broadinstitute.org) or NHLBI Trans-Omics for Precision Medicine program[63] (https://www.nhlbi.nih.gov/research/resources/nhlbi-precision-medicine-initiative/topmed),which variants cause amino acid changes or generate a stop codon, which fall within regions of DNA copy number variations[64] or conserved regions among numerous species.[65] Annotation tools are also able to predict the pathogenicity of variants by bioinformatics tools such as SIFT[66] and PolyPhen2,[67] which predict the effect of an amino acid substitution on the structure and function of a protein using sequence homology; and by query clinical catalogues, including the Human Gene Mutation Database,[68] the Online Mendelian Inheritance in Man,[69] ClinVar,[70] and Catalogue of Somatic Mutations in Cancer,[71]with the aim to highlight variants that are known to be associated with certain phenotypes and/or diseases. Prioritization methods are also useful in the research setting, to discover clinically relevant variants or genes. Tools such as eXtasy,[72] ToppGene,[73] and SUSPECTS[74] are designed to identify, among a user-defined gene list, those that are more likely associated with a certain disease, by exploiting the integration of heterogeneous datasets, such as literature reports, expression, and functional data. According to these criteria, variants can be classified as pathogenic, likely pathogenic, variants of uncertain significance (VUS), or benign.

Functional assays

A reliable functional assay is generally recommended to verify the biological effect of an unknown genetic variation. Patch clamp is the gold standard in studying the electrophysiologic properties of mutated versus wild-type cardiac channels.[75,76] Cardiomyopathies provide a particular challenge to establish causality for putative disease-causing variants, because of the complexity to assay the effect of genetic variants in human cardiac structural proteins. Varieties of in-vitro and in-vivo techniques have been used to verify the function of mutant structural macromolecules.[77–80] The stringency for emphasizing relevant pathologic changes in an appropriate model is addressing to use the induced pluripotent stem cells to create an investigational tissue source from a study participant harbouring the variant that has the same genetic background.[81,82] However, these techniques are labour intensive and require highly skilled staff, therefore are not readily applicable to large-scale approaches and not feasible in a diagnostic setting.

Genetic counselling

The general goals of genetic counselling are to increase patients' knowledge and awareness about their disease and its genetic aspects, and to ensure that patients can control their feelings about their condition, resulting in the ability to make autonomy choices for themselves and their relatives. Discussions with the patient (and the parents in the case of children) about the importance

of genetic information for their kindred, as well as a recommendation that information be shared with potentially affected family members as appropriate, is a standard part of genetic counselling.

Genetic evaluation is indicated in paediatric and adult patients with signs of systemic diseases, including facial dysmorphisms, skeletal and cutaneous abnormalities, mental retardation, delayed speech, sensorineural deafness, skeletal myopathy, diabetes, to exclude genetic syndromes, metabolic/infiltrative diseases, mitochondrial, and neuromuscular disease, as a cause of cardiomyopathies.

Informed consent

The process of educating a person about the test and obtaining permission to carry out testing is called informed consent. 'Informed' means that the person has enough information to make an educated decision about testing; 'consent' refers to a person's voluntary agreement to have the test done. In general, only adults who are competent to make medical decisions for themselves can give informed consent. For children and others who are unable to make their own medical decisions, either parents or legal guardian must take responsibility. The genetic counsellor, or trained healthcare professionals, discusses the test, answers the questions, and obtains the consent. Several factors are commonly included in the consent form: the general description of the test, including the purpose and the condition for which the testing is being performed; the biological sample required for the analysis (for example, a blood sample);what the test results mean, including positive and negative results, and the potential for uninformative results or false positives or false negatives results; whether the results might provide information about other family members' health, including the risk of developing a particular disease or the possibility of having affected children; how and to whom test results should be reported; what will happen to the test specimen after the test is complete; and acknowledgement that the person requesting testing has had the opportunity to discuss the test with a healthcare professional. Furthermore, the advent of new techniques, such as clinical WES, raises the problem of incidental findings, that is, genetic results that you aren't looking for. Therefore, the patient must be informed of this possibility and should express his/her will whether to know or not such results. The patient and the counsellor must sign the informed consent. It is important to remind patient that, even after signing, he may still opt out at any time, and that the informed consent document is not a binding contract.

Genetic counselling in structural Cardiomyopathies

HCM, DCM, ARVC, and left ventricular noncompaction are the most frequent structural cardiomyopathies for which genetic test can be proposed as part of the diagnostic flow chart. The main genes involved in HCM, DCM, and ARVC are summarized in Table 1. Inherited cardiomyopathies generally show an autosomal dominant, or less frequently an autosomal recessive or X-linked pattern of inheritance and are characterized by a large variable expressivity and age-dependent penetrance. Genetic test is recommended even in patients with no family history of inherited cardiomyopathies or SD, as this may simply reflect inaccuracies of family history and screening, incomplete penetrance, or a de-novo mutation in proband. In all structural cardiomyopathies risk of transmission is 50% at each pregnancy and the knowledge of the disease-causing mutation is crucial for family planning even though inheritance probability does not reflect 'the risk' of having the disease.

HCM is reviewed here as a paradigm of inherited cardiomyopathies, although most considerations with regard to gene testing also apply to the other conditions. It is the most common monogenic cardiac disorder and a prevalent cause of sudden cardiac death in young people and competitive athletes.[15,83,84] The first-tier genetic test for HCM patients includes the most commonly implicated sarcomere protein genes (*MYBPC3*, *MYH7*, *TNNT2*, *TPM1*, *MYL2*, *MYL3*, *TNNI3*, and *ACTC1)* with a diagnostic sensitivity of about 65% (Table 1).[22] Patients presenting features suggestive of specific genetic subsets (i.e. Anderson–Fabry, Danon, Noonan/LEOPARD, Friedreich's ataxia, amyloidosis, mitochondrial diseases, etc.) or that are negative at first-tier test, are candidates to further testing to exclude rare phenocopies.

International position statements recommend genetic test in HCM as a class I indication, based on the potential clinical benefit and favourable cost-efficacy profile.[22] The main benefits of genetic testing for the proband include the possibility of achieving a definitive diagnosis and the identification/exclusion of few high-risk mutations or complex genotypes (multiple mutations). These genotypes are usually associated with severe disease expression, such as marked hypertrophy, premature heart failure, and progression to the hypokinetic restrictive stage.[85] A recent meta-analysis based on a comprehensive genotype–phenotype analysis reveals that HCM patients show an earlier age at onset and a more severe phenotype compared with patients without such mutations. Furthermore, patients with sarcomeric mutations are more susceptible to SCD in comparison with HCM patients without sarcomere mutations. Although the great clinical variability, even within families, suggests that therapeutic choices should not be based on genotype, it seems reasonable to include genetic findings in risk assessment, especially in patients with borderline risk for SCD by conventional clinical scoring systems.[86]

A second-tier test, based on extended gene panels, may distinguish rare HCM phenocopies, which need to be

Table 1 Main genes involved in cardiomyopathies (hypertrophic cardiomyopathy, dilated cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy)[a]

Hypertrophic cardiomyopathy

Sarcomeric genes/phenocopy genes

	Gene	Frequency (%)
β-myosin heavy chain	*MYH7*	20–30
Cardiac myosin-binding protein C	*MYBPC3*	30–40
Regulatory myosin light chain	*MYL2*	2–4
Cardiac troponin T	*TNNT2*	3–5
Cardiac troponin I	*TNNI3*	<5
α-tropomyosin	*TPM1*	<1
α-cardiac actin	*ACTC1*	<1
Essential myosin light chain	*MYL3*	<1
Galactosidase, α	*GLA*	<1 Fabry disease
Lysosomal-associated membrane protein 2	*LAMP2*	<1 Danon disease
Protein kinase, AMP activated, γ2 subunit	*PRKAG2*	<1 Wolff–Parkinson–White syndrome

Dilated cardiomyopathy

Sarcomeric/Zdisc genes

	Gene	Frequency (%)
Titin	*TTN*	15–25
β-myosin heavy chain	*MYH7*	3–4
Cardiac troponin T	*TNNT2*	3
α-tropomyosin	*TPM1*	1–2
α-cardiac actin	*ACTC1*	<1
Cardiac troponin I	*TNNI3*	<1
Cardiac troponin C	*TNNC1*	<1
?-actinin 2	*ACTN2*	<1
Telethonin	*TCAP*	<1
Cardiac ankyrin repeat protein	*ANKRD1*	<1
Cypher/ZASP	*LDB3*	<1
Muscle LIM Protein	*CSRP3*	<1

Other genes (cytoskeletal/desmosomal/nuclear envelope/dystrophin complex/nucleus/ion channels/sarcoplasmic reticulum, and cytoplasm)

	Gene	Frequency (%)
Lamin A/C	*LMNA*	4–8
Type V voltage-gated cardiac Na Channel	*SCN5A*	2–3
Desmoplakin	*DSP*	2
RNA-binding protein 20	*RBM20*	2
Metavinculin	*VCL*	1
Filamin C	*FLNC*	1
Dystrophin	*DMD*	<1
Desmin	*DES*	<1
Sulfonylurea receptor 2A	*ABCC9*	<1
Δ-Sarcoglycan	*SGCD*	<1
Phospholamban	*PLN*	<1

Arrhythmogenic right ventricular cardiomyopathy

Desmosomal genes/other genes (nuclear envelope/intermediate filament/growth factor)

	Gene	Frequency (%)
Plakophilin-2	*PKP2*	30–40
Desmoglein-2	*DSG2*	5–20
Desmoplakin	*DSP*	10–20
Desmocollin-2	*DSC2*	1–2
Junction plakoglobin	*JUP*	1–2
Transmembrane protein 43	*TMEM43*	<1
Transforming growth factor 3	*TGFB3*	<1
Desmin	*DES*	<1
αT-catenin	*CTNNA3*	<1
Cadherin C	*CDH2*	<1

[a] OMIM (www.omim.org); GeneCards (www.genecards.org).

diagnosed at an early stage. Several clinical red flags may suggest an alternative diagnosis to 'classic' HCM. These include an X-linked or autosomal recessive pattern of inheritance, peculiar ECG signs, extracardiac manifestations, and so on. Diagnosis is important because of management implications (e.g. availability of enzyme replacement therapy in Fabry disease and early need for cardiac transplantation in Danon disease). Once a causative mutation is found in the proband, genetic testing of first-degree family members, leading to cascade genetic screening, is strongly indicated (class I), to promote preclinical diagnosis and prevention in affected family members and implement follow-up. Relatives found not to carry the mutation do not require further clinical workup, provided that the causative role of the mutation is well established.

Genetic counselling in channelopathies

LQTS, BrS, CPVT, and SQTS are the main channelopathies for which expert consensus documents have been published, providing clear recommendation on when to perform a molecular screening as part of the diagnostic assessment and on which genes.[15,87] These recommendations are based on two major considerations, the impact of genetic testing on clinical management and the yield of the test (that for clinical purposes should be focused exclusively on the major genes). These two concepts are also at the basis of a good genetic counselling. In probands with LQTS and CPVT, genetic testing has a class I indication and a disease-causing mutation can be identified in 70–80% and 60–70% of probands, respectively.[15,88] The identification of a disease-causing mutation should be perceived positively by an adequately informed patient, as a means to improve clinical management. This is impressively shown in LQTS, because of the fact that arrhythmic triggers, response to therapy and prognosis differ based on the disease-causing gene[89] and sometimes to the specific mutation.[90] As an example, patients with a *KCNQ1* mutation (LQT1 patients) are at higher risk during physical activity, but are very well protected by β blockers; whereas patients with *KCNH2* mutations (LQT2 patients) are known to be at higher risk in the presence of sudden noises and in the postpartum period, and their response to β blocker therapy is reasonably good.[89] As a consequence, LQT2 are advised not to keep telephones or loud alarm clocks making loud noises in the bedroom and, in the case of a women LQT2 mother, we prompt the father to take care of nocturnal parental duties. In LQT3 harbouring a *SCN5A* mutation, a gene-specific therapy with sodium channel blockers may be considered in addition to β blockade.[91] Furthermore, specific genes, as genes encoding for the calmodulin protein (*CALM* 1–3), are associated with a very severe phenotype and poor response to available therapies.[92] At variance with LQTS and CPVT, in SQTS a disease-causing mutation is identified in less than 5–10% of probands and the impact on the clinical management

is limited. Therefore, genetic testing is a class IIb recommendation. In these conditions BrS represents and intermediate situation, with class IIa recommendation.[15]

A very important issue that should be discussed with the patient before genetic counselling is the impact of the genetic test result on the clinical management of the entire family. In all channelopathies, as in structural cardiomyopathies, risk of transmission is 50% at each pregnancy and the knowledge of the disease-causing mutation could theoretically allow the selection of the unaffected embryos. However, this information, which clearly has a strong ethical impact, should be given together with the risk of the disease in children and adults and with the information about how treatable the disease is and which is the impact of the disease on a growing child. The identification of a disease-causing mutation in the proband should trigger cascade testing in the whole family (class I indication for all channelopathies).

When, where, and how to perform genetic testing

When

Genetic testing should be offered to index patients who fulfil diagnostic criteria for genetic cardiovascular disease. A comprehensive clinical evaluation should precede genetic testing, as a precise clinical diagnosis (or reasonable suspicion) is extremely important in guiding the type of test on which needs to be performed. As discussed, testing is recommended in family members only when a gene mutation disease-causing mutation has been already identified.[93] Careful consideration is needed when family members are asymptomatic children or adolescents.[93] Genetic testing is recommended in children under the age of 4 years on in families with channelopathies and after the age of 10 years in families with structural progressive cardiomyopathies, unless conditions of anxiety because of uncertainty, and the need of a realistic lifestyle planning and clinical follow-up might advise earlier testing.[22]

In the recent years, there has been an increasing emphasis on the role of genetic testing of DNA obtained at autopsy (also called 'molecular autopsy'). In this setting, pathologists play an important role in the identification of families with hereditary conditions, by reporting whether it is recommended to refer first-degree family members for clinical screening and/or to perform additional postmortem genetic testing. According to the European Society of Cardiology guidelines,[94] targeted postmortem genetic analysis of potentially disease-causing genes should be considered in all SD victims in whom a specific inheritable channelopathy or cardiomyopathy is suspected (class of recommendation IIa and level of evidence C). However, the SD guidelines of the Association for European Cardiovascular Pathology recommend that preliminary genetic counselling of family members is carried out before performing postmortem genetic testing (Basso *et al.* Virchow Arch 2017 submitted). Furthermore, the problem of costs, not yet supported by the NHS for a dead person in Italy, is still a major issue.

Where?

The complexity of diagnosing inherited cardiovascular diseases highlights the importance of dedicated cardiogenetic services. DNA testing should be performed in certified laboratory and counselling should be performed by trained healthcare professionals, working within multidisciplinary teams to help patients understand and manage the psychological, social, professional, ethical, and legal implications of genetic disease. Information should be provided on existing legislative protection for discrimination based on genetic testing, including discussion of areas that are not protected. These laws vary from country to country. Psychological support can be provided, especially with posttest counselling, to help individual scope with anxiety associated with the disease or genetic result. A comprehensive evaluation of patients and families should be undertaken in referral institutions providing the expertise of cardiologists, electrophysiologists, radiologists, geneticists, pathologists, psychologists, and molecular biologists as well as experts in bioinformatics, ethics, and health services research. An interdisciplinary team with synergistic areas of expertise will ensure clinical diagnosis, provide optimal counselling, and management of families with hereditary cardiovascular conditions. The management of patients with cardiovascular diseases includes expert judgement regarding indications, type, and interpretation of genetic testing.

How?

In general, genetic testing are a free choice of patients and are never a mandatory step in the clinical management of the patients. Pretest counselling is necessary to provide patients with the relevant information (benefits and limitations of the test and the possible consequences of the test results) and generate realistic expectations allowing a free and aware choice of the patient. Moreover, ~~pretest~~ counselling is essential to accurately collect information on family and clinical history and to assess the presence and severity of risk factors. At ~~posttest~~ counselling, genetic results should be discussed directly with the patient in presence of a cardiologist and a geneticist. ~~Postcounselling~~ has the main objective to help clinicians to interpret results based on familial and clinical evidences.

How to interpret a genetic result

The results of genetic testing can be complex. Although a result may be categorized as positive, negative, or uncertain, its clinical significance strongly depends on the patient's personal and family history, and the clinical context should never be forgotten when interpreting the variants identified.

Table 2 When, where, how to perform genetic testing in patients/families affected by inherited cardiac disease

When	Where	How
Genetic testing should be offered to index patients who fulfil diagnostic criteria for familial cardiovascular disease	In dedicated cardiogenetic services	Genetic tests are usually performed on DNA extracted ~~for~~ a blood sample
Probands with a precise clinical diagnosis (or reasonable suspicion) Family members only when a gene mutation has been already identified Careful consideration is needed when family members are asymptomatic children or adolescents	DNA testing should be performed in certified laboratory Counselling should be performed by trained healthcare professionals working within multidisciplinary teams	~~Pretest~~ counselling should be offered to: draw family pedigrees, collect information on family history, and help patients comprehend the procedure, benefits and limitations of the test and the possible consequences of the test results ~~Posttest~~ counselling should be offered: to discuss genetic results directly with the patient in presence of a cardiologist and a geneticist

Positive results

If the genetic test result is positive, this means that a rare variant compatible with the presenting diagnosis was identified and that its pathogenicity is clear and well documented. If the purpose was to diagnose or confirm the genetic aetiology of a specific disease or condition, a positive result will help to determine the right treatment, management, and follow-up plan. If the aim of genetic testing was to find out if an individual is carrying an altered gene that could cause disease (presymptomatic and predictive testing), and the test is positive, the risk of actually developing the disease will depend on multiple factors, many not yet known, and frequently different according to the specific inherited cardiac disease. In other words, in the case of cardiomyopathies a positive test does not necessarily mean that the study participants will manifest the disorder. For example, having a familial mutation in HCM gene means that there is a high risk of developing the disease at some point in life, but neither its actual occurrence nor its severity may be predicted. In case of channelopathies, having the genetic defect means that the disease is there and therefore preventive strategies should be established. For example, having a familial mutation in a LQTS gene, means being at higher risk of SCD even if the basal ECG shows normal values. Therefore, lifestyle changes should be recommended and β blocker therapy may be indicated.

Negative results

A negative result in the probands means that a genetic alteration was not detected by the test. But a negative result does not guarantee that the disease is not genetic. The accuracy of genetic tests to detect alterations varies depending on the condition being tested for. Genetic testing may not be able to detect all genetic defects causing a genetic disease as this is an evolving field. A negative result today may be positive in the next future.

On the other hand, in family members being found not to carry the harmful gene alteration, previously identified in their family, the risk to develop the hereditary cardiomyopathy may be virtually excluded: they may feel less anxious, have a better quality of life and may also benefit from the knowledge that they have not passed the gene alteration onto their children.

Inconclusive results

In some cases, a genetic test may not be able to provide helpful information about the genetic aetiology of the disease. Everyone has variations in a multitude of genes, and very often, these variations do not affect health. Sometimes it can be difficult to distinguish between a disease-causing gene alteration from 'the background noise', the so called VUS, which means that although the testing laboratory detected a DNA alteration, there was not enough evidence to classify that alteration as deleterious or neutral, therefore the variant has an uncertain clinical significance. In these situations, follow-up testing may be necessary, such as the clinical and genetic characterization of other family members. The VUS classification is the subject of international efforts, although there remain no universally accepted methods to establish pathogenicity and to report VUS results. The progression of knowledge, the continuous increase in related disease variants worldwide, the availability of largest families, the ability to perform functional studies, and so on can modify the actual weight of a single variant in the causal relation with a specific disease. Moreover, a VUS should not be used in clinical decision-making before follow-up testing is completed (Table 2).

Ethical issues in genetics: bioethics considerations

There are several aspects of genetic testing that may lead to ethical dilemmas, some inherent to the characteristics of a specific test (e.g. the limitations of what genetic testing can provide in specific clinical situations) other inherent to genetic condition/results (privacy, biological identity, familial implications, etc.). In general, genetic testing enhances phenotypic screening and clinical surveillance, and for any clinical purpose should be tied to the availability of intervention, including counselling, lifestyle changes, reproductive decision-making, and prenatal diagnosis. The physician ordering genetic test has the responsibility to use it correctly. Therefore, it is preferred that the patient refers to a geneticist with experience in the field of cardiomyopathies or channelopathies, who is aware of when it is appropriate to test and which particular test to order, what information the

test can provide and what limitations testing presents, how to interpret positive, negative, and uncertain results in light of the patient's medical or familial history. The geneticist may also suggest in-depth testing, when necessary and based on the progress of technology and scientific knowledge. Testing of children presents unique issues in counselling and consent. In general, there are two extreme situations, one when the child is suffering from a genetic disease and genetic testing enters the path to achieving a diagnosis (diagnostic testing); the other when the child is apparently healthy but belongs to a family with a genetic disease that manifest mainly, but not always, in adulthood (predictive testing). The latter case opens ethical dilemmas and the physician should balance the rights of the parents to have information that can optimize the ongoing healthcare of their children against the rights of the children to have their best interest protected. The fundamental problem is implicit in the genetic characteristics of these diseases (variable penetrance and expressivity) and, consequently, in low predictive power *per se* of the genetic test. In this scenario, integration between clinical data (i.e. age at onset and severity of symptoms in the relatives), family history (positive or negative for sudden death) and genetic results (presence of specific 'malignant' mutations) can drive the choice. If the child belonging to a cardiomyopathy – or channelopathy – family enrols in a competitive sport, the genetic test should be strongly considered.

Conclusion

Molecular cardiology has become an important tool to study and understand the aetiology, pathogenesis, and development of familial cardiomyopathies and channelopathies and is beginning to change clinical practice. Advances in contemporary DNA sequencing methodology have made gene-based diagnosis increasingly feasible in routine clinical practice, but this should not occur at the expense of clinical accuracy. Future efforts should be aimed at promoting awareness of inherited cardiovascular diseases among community-based cardiologist and primary care physicians, as well as establish high-standard multidisciplinary referral teams on a regional and national level, to guarantee the best possible use of genetic testing in patients and their families.

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Conflicts of interest

None declared. AQ6

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